REMARKS

Applicants thank the Examiner for the courtesy extended to Applicants' attorney during the interview held September 12, 2003, in the above-identified application. During the interview, Applicants' attorney explained the presently-claimed invention and why it is patentable over the applied prior art, and discussed other issues raised in the Office Action. The discussion is summarized and expanded upon below.

This invention relates to a pretreatment method for accurately and efficiently discriminating and quantitating cholesterol, which exists in the specific lipoprotein fraction, by simple procedures while using a small amount of a sample, and also to a method for measuring cholesterol in the specific lipoprotein fraction by using the pretreatment method.

As described in the specification under "Background Art," beginning at page 1, second paragraph, many methods are known in the art for measurement of cholesterol in various lipoproteins. Various of these methods involve the use of a precipitation reagent, but such reagents are problematical because they result in turbidity. Various solutions to the problem of turbidity have been suggested, but these solutions require the use of a precipitation reagent such as a polyanion.

The present invention seeks to accurately quantitate cholesterol without using a precipitation reagent, and without needing a polyanion or the like.

Applicants investigated the cause of a problem in connection with earlier work in which a value of cholesterol in a specific lipoprotein fraction as quantitated by using a substance which acts only upon the specific lipoprotein such as HDL, becomes higher than the corresponding value as determined by the precipitation method, and concluded that even from non-HDL lipoproteins such as LDL, VLDL and the like, the cholesterol of which is not supposed to be measured, a small amount of free cholesterol existing on their surfaces or in the vicinity of their surfaces is liberated to cause a positive error. Based on this finding,

Applicants found that a cholesterol value obtained by a quantitation method making use of a substance, which acts upon a specific lipoprotein only, becomes consistent with the corresponding value obtained by the precipitation method when the cholesterol value is measured after converting only free cholesterol in advance, under conditions that lipoproteins remain substantially unchanged, leading to the present invention. Thus, in the present invention, a sample containing various lipoproteins is pretreated in order to convert the free cholesterol without substantially changing the remaining lipoproteins.

The pretreatment involves the action of an enzyme on the free cholesterol. Applicants have also discovered that the amount of enzyme may be lowered when used in combination with a reaction accelerator, such as those described in the specification at page 10, last paragraph. When the enzyme is used in the same amount with the reaction accelerator, on the other hand, the reaction accelerator can shorten the reaction time, as described in the specification at page 11, third paragraph.

The rejection of Claims 2, 5, 36, 37 and 40 under 35 U.S.C. § 101 as inoperative and lacking utility, is respectfully traversed. The Examiner's premise is that Applicants have not shown that the above-discussed function of the reaction accelerator is actually carried out. Rather, the Examiner has analyzed data in the specification and concludes that the reaction accelerator does not perform as described.

In reply, and as Applicants' attorney pointed out at the above-referenced interview, while Applicants will explain below why the Examiner's analysis is incorrect, nevertheless, the Examiner has applied the wrong standard for operability and utility. The issue under 35 U.S.C. § 101 is whether the claimed invention has utility, not whether aspects of the invention perform as described. Thus, even if the Examiner were correct that the reaction accelerator does not perform as described, there is no basis for holding that the claimed method would not act as recited in the claims.

As discussed above, the Examiner analyzed data in the specification. This data is from Table 5 of Example 4. A copy of Table 5, which is found at page 35 of the specification, is **attached herewith**. Particularly, the Examiner found that no statistically significant differences are shown from the measurement value on the standard test system even when the concentration of cholesterol oxidase is reduced to one-fifth of the standard concentration. The Examiner concluded that use of a reaction accelerator has not been fully substantiated as effective to avoid a decrease in measurement value which would otherwise occur when cholesterol oxidase is used at a lower concentration. The Examiner's conclusion is based on failure to correctly understand this data, as now described.

In the experiment of Example 4, various test systems were designed, including a test system (Test System A) in which the concentration of cholesterol oxidase was reduced to one-fifth compared with the standard system and test systems (Test Systems B to I) in each of which cholesterol oxidase was added at a one-fifth concentration compared with the standard system together with the corresponding reaction accelerator. Using those test systems, the cholesterol in HDL in each of 25 common samples was quantitated. The measurement values so obtained are shown in Table 5.

Concerning each of the test systems, the measurement values were compared with those obtained by the precipitation method which is considered to be a standard method. The measurement results expressed in terms of correlation coefficient, slope and intercept are also given in the last three lines of the Table.

The above-discussed Test System A and Test Systems B to I each demonstrated an extremely high correlation with the precipitation method as readily envisaged from their correlation coefficients.

According to the regression equation between the standard system and the precipitation method, however, the intercept, which should theoretically be 0, is 5.6.

According to the regression equation between the Test System A and the precipitation method, on the other hand, the intercept is 8.7. As a lower concentration of cholesterol oxidase results in a higher intercept, these deviations in intercept are considered to be attributable to the detection of a small amount of free cholesterol existing on or in the vicinity of surfaces of non-HDL lipoproteins (LDL, VLDL and the like) the cholesterol of which is not supposed to be measured.

The Test Systems B to I (the invention method) all gave intercepts smaller than the intercept (8.7) of the Test System A (in other words, linear lines as expressed by the regression equations, were drawn under that of the Test System A), although the Test Systems A to I contained cholesterol oxidase at the same concentration (1 U/mL) which was one fifth compared with the concentration of cholesterol oxidase in the standard system. This indicates that with each of the Test Systems B to I, the accelerator accelerates the conversion of free cholesterol in pretreatment and reduces the effect of the free cholesterol on the measurement result.

Stated another way, if there is no free cholesterol before the addition of a second reagent, the intercept should be 0 because the reaction takes place only with cholesterol from HDL. However, free cholesterol exists at this stage so that a positive error occurs on the intercept. Therefore, a greater error occurs especially when cholesterol oxidase is used at a lower concentration.

In Test Systems B to I, on the other hand, the intercepts are all smaller than the intercept in Test System A although in each of Test Systems A to I, the concentration of cholesterol oxidase was one-fifth of its concentration in the standard test system.

This indicates that each of the reaction accelerators employed in Test Systems B to I contributes to the decomposition of free cholesterol which exists before the addition of the

second reagent, and further that use of such a reaction accelerator makes it possible to provide a method which features smaller errors.

The Examiner's indication that no statistically significant differences arose from the measurement value on the standard test system is believed to relate to correlation coefficient. This also means that the use of the above-described reaction accelerator can provide a measurement system without any problem. If one or more of the measurement values included those having statistically significant differences, this would indicate that use of the corresponding reaction accelerator(s) would be able to provide no useful measurement system(s).

As explained above, Table 5 in Example 4 indicates that the reaction accelerators used in Test Systems B to I contribute to the decomposition of free cholesterol.

It is appreciated from FIG. 3 in Example 5 that, although the relative absorbance decreases as the concentration of the enzyme (cholesterol oxidase) becomes lower, the addition of flufenamic acid as a reaction accelerator makes the relative absorbance increase depending on the concentration of flufenamic acid.

For example, see the top graph in FIG. 3, which relates to a measurement system of pH 6.0. The graph indicates that, even when the concentration of the enzyme (cholesterol oxidase) is 0.5 U/mL, use of 0.1mM flufenamic acid can bring about the same effect as the addition of the enzyme at 5 U/mL.

This means that flufenamic acid as a reaction accelerator accelerated the enzyme reaction.

For all the above reasons, it is respectfully requested that this rejection be withdrawn.

The rejection of Claims 1, 3, 4, 6, 7, and 38 under 35 U.S.C. § 102(a) as clearly anticipated by JP 11-155595 (JP '595), is respectfully traversed. (The rejection of Claim 38 appears to be in error, since Claim 38 depends on Claim 2, not subject to this rejection.)

JP '595 discloses a method for determination of lipoprotein cholesterol comprising treating the cholesterol of non-target lipoproteins with cholesterol oxidase, measuring light absorbance, treating cholesterol of the target lipoprotein with cholesterol dehydrogenase, measuring light absorbance, and determining the difference between the former absorbance and the latter absorbance. The enzyme treatment is carried out in the presence of compounds forming water-soluble complexes with cholesterol to prevent formation of aggregates. JP '595 neither discloses nor suggests the presently-claimed subject matter. Accordingly, it is respectfully requested that this rejection be withdrawn.

The rejection of Claims 1-7 and 36-40 under 35 U.S.C. § 112, second paragraph, is respectfully traversed. The term "consumed" has been replaced with --converted--. The term "consuming" or "consumed" was used in the specification to mean that an enzyme which acts upon free cholesterol as a substrate is caused to act as pretreatment on a sample to convert free cholesterol to a different sterol such that the free cholesterol can no longer take any part in the next step, i.e., in the system for quantitating cholesterol in a particular lipoprotein. It is respectfully submitted that one skilled in the art would have understood the original meaning of the above-quoted term. Nevertheless, it is respectfully submitted that the above-discussed amendment now moots this issue. During the above-referenced interview, the Examiner appeared to agree.

The Examiner also finds that Claims 36 and 37 are indefinite because no sample is mentioned upon which the enzyme "acts" and no quantitating step is seen, among other critiques. Nevertheless, the issue is now moot in view of the cancellation of these claims and replacement with new Claims 43 and 44.

For all the above reasons, it is respectfully requested that this rejection be withdrawn.

Applicants respectfully call the Examiner's attention to the Information Disclosure Statement (IDS) filed April 16, 2003. The Examiner is respectfully requested to initial the

Form PTO-1449 submitted therewith, and include a copy thereof with the next Office communication.

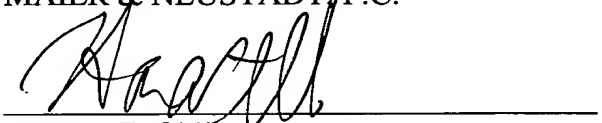
Moreover, since the date of the IDS is before the date of the Office Action and thus technically was part of the Official File as of the Office Action date, Applicants respectfully request that should the Examiner determine that a new ground of rejection needs to be made in the next Office Action, relying in whole or in part on any of the references cited in the IDS, then said next Office Action not be made Final, even if the new rejection was necessitated by the present amendment to the claims.

Finally, Applicants note the acknowledgement in item 10 on the Office Action Summary page of the drawings filed December 21, 2002, but does not indicate whether the drawings are accepted or objected to. Applicants acknowledge the Examiner's indication during the above-referenced interview that the drawings have been accepted, as reflected on the corresponding Interview Summary.

All of the presently pending and active claims in this application are now believed to be in immediate condition for allowance. Accordingly, the Examiner is respectfully requested to pass this application to issue.

Respectfully submitted,

OBLON, SPIVAK, McCLELLAND,
MAIER & NEUSTADT, P.C.



Norman F. Oblon
Attorney of Record
Registration No. 24,618

Harris A. Pitlick
Registration No. 38,779

Customer Number
22850

Tel: (703) 413-3000
Fax: (703) 413 -2220
(OSMMN 08/03)
NFO/HAP/cja

Table 5

Sample	Precipitation method (mg/dL)	Test system (mg/dL)									
		Standard	A	B	C	D	E	F	G	H	I
1	80	77	72	77	68	76	74	74	74	74	76
2	76	74	72	74	64	73	71	73	74	74	73
3	75	72	70	72	66	71	70	70	70	71	71
4	71	72	71	70	66	69	69	71	69	71	72
5	71	70	70	69	61	68	67	68	70	69	70
6	71	70	67	70	63	68	68	67	69	70	68
7	69	66	63	66	61	65	65	65	64	65	66
8	67	69	70	68	60	68	67	66	69	68	68
9	66	65	65	65	59	65	64	63	65	65	65
10	65	65	64	65	58	65	64	62	64	65	63
11	57	58	56	57	54	57	56	57	57	58	57
12	56	56	55	55	49	55	54	53	55	55	55
13	54	55	54	55	50	54	53	53	53	54	54
14	53	54	54	52	46	53	52	52	54	52	53
15	52	53	52	51	47	52	51	49	52	51	52
16	51	53	51	50	46	50	51	49	51	51	51
17	49	50	48	48	44	47	48	47	48	48	49
18	47	48	48	46	41	46	46	45	47	47	47
19	45	46	48	44	38	46	43	45	47	47	46
20	47	47	49	45	40	46	45	45	48	47	47
21	42	44	44	43	39	43	42	41	43	44	43
22	39	42	43	41	37	41	41	39	41	41	41
23	32	35	36	33	31	36	34	32	34	34	33
24	18	20	22	19	17	23	19	18	21	20	19
25	40	42	42	41	38	45	41	40	41	42	41
Correlation coef.	-	0.996	0.990	0.998	0.992	0.995	0.997	0.997	0.994	0.995	0.997
Slope		0.905	0.838	0.941	0.832	0.856	0.888	0.915	0.877	0.891	0.917
Intercept		5.6	8.7	2.6	3.4	7.5	4.6	2.8	6.3	5.7	4.1